VOLUME XLI NUMBER 4

BOTANICAL GAZETTE

APRIL, 1906

CYTOLOGICAL STUDIES ON THE ENTOMOPHTHOREAE.

II. NUCLEAR AND CELL DIVISION OF EMPUSA.

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(WITH PLATE XVI[1])

THE division of the nuclei in Empusa has been found in the course of this investigation to resemble closely that described for Amoeba, Euglena, and other Protozoa. Such a primitive type of nucleus, which has been regarded as the typical protozoan form, has not so far been observed in the Metazoa, nor have any of the lower plants heretofore revealed a type of nucleus in which the "division-center" is permanently intranuclear. Such a type has been called by BOVERI (:00, p. 183) a "centronucleus," since it contains within itself a center of division which he assumes may be either in diffuse or concentrated form.

The varieties of protozoan nuclei and the types into which they may be conveniently grouped are discussed by WILSON (:00), by CALKINS (:01), and at some length by CALKINS in a recent article (:03); hence we may concern ourselves here mainly with those forms which appear to show nuclear conditions nearest those in Empusa.

SCHAUDINN published in 1894 an account of the division of the nucleolus-like body in the center of the dividing nucleus of Amoeba, and although he recognized that this appeared to play the chief rôle in nuclear division, he reserved, till further comparative studies, his ideas on the mechanical details of the process.

[1]As in this paper I shall have to refer frequently to the figures already published in *plates XIV* and *XV* which accompanied my foregoing paper on *The morphology and development of Empusa* (BOT. GAZETTE 41: 192–208. March 1906), I have numbered the figures on this plate consecutively with them.

BLOCHMANN ('94) and KEUTEN ('95) first described the division of the centronucleus in Euglena, and the latter author gave an interpretation of the function of the nucleolus, giving to it the name "nucleolo-centrosoma" (p. 219). According to KEUTEN's observations, the nucleolus-like body of Euglena elongates in the prophases of nuclear division, and functions as a kind of spindle, which, however, appears to be solid and homogeneous, and not fibrillar as in the usual type of spindle. Other spindle substance and centrosomes, as well as "pole-bodies," the author could not find. The chromatin forms many chromosome-like bodies, which, after passing through an "equatorial ring" stage, are finally arranged in diverging daughter groups about the elongated axial strand of the nucleolo-centrosome. Just what the relation is between the dumb-bell shaped nucleolo-centrosome and the dividing chromosomes is not made clear in KEUTEN's figures, although he asserts that this axial rod governs the entire process of nuclear division, since it orients the plane of division and since the chromosomes move along it. Whether this intranuclear body functions solely as an active fibrous mechanism for separating the chromosomes, or whether its poles have in addition the properties of centrosomes, are matters which should be more clearly determined before we can make comparisons with the conditions in Empusa.

BOVERI (:**00**, p. 182, note) suggests in this connection that the nucleolo-centrosome of Euglena is probably a concentrated and sharply individualized intranuclear spindle. CALKINS (:**01**, p. 265) further points out what he regards as an analogy existing between such a connecting rod in Euglena and the true fibrous spindle seen in higher forms.

SCHAUDINN (:**00**) has described a type of nuclear division in the sporozoan, *Coccidium shubergi*, parasitic in the intestine of a myriapod, which resembles even more closely that of Empusa. In the growing individual, according to this author, the nuclei divide a number of times, and finally, by a process resembling progressive cleavage, the body is cut up into many uninucleate individuals, which he terms merozoites. The division of the nucleus at this time is by a "primitive mitosis" (p. 230), totally unlike the double division which takes place in later stages, following the fertilization of the egg. The division in the first instance is quite similar to that of Amoeba and

Euglena, and also resembles very closely that in Empusa. The second kind of nuclear division is regarded by SCHAUDINN as still simpler, since centrosomes appear to be wanting entirely. The close resemblance of the latter type to certain division-figures in Empusa suggests, however, that the differences noted by SCHAUDINN may have been more apparent than real, and that poor fixation, due perhaps to the thickness of the membrane about the fertilized egg, may have been the cause for his failure to find the intranuclear centers in these cases also.

According to SCHAUDINN (p. 229), in the first mentioned division the chromatin granules gather in the center of the primary nucleus about a diffuse, slightly refractive substance, which stains less with haematoxylin than the chromatin. There results finally a globular central body, which he calls a karyosome, made up of two substances, plastin and chromatin. Upon the appearance of vacuoles within it, the karyosome grows larger, and it ultimately elongates to form a dumb-bell shaped central core to the dividing nucleus. At this stage the chromatin strands appear to radiate from the poles of the central body, differing in this respect from the corresponding nuclear figure of Euglena. The continued elongation of the central core is accompanied by the further massing of chromatin about the two daughter-halves of the central body, and the nucleus finally assumes a shape comparable to an hour-glass. In the slender connecting strand which unites the diverging nuclear halves there appears a peculiar *Zwischenkörper* which SCHAUDINN regards as probably a thickening of the fibrous strand which connects the halves of the karyosome. After the final constriction into two, the daughter nuclei, without entering upon a period of rest, begin immediately a second division.

While those members of the Entomophthoreae which live parasitically in the bodies of insects have attracted attention for more than a century, only a few investigators have published observations on the coenocytic character of the mycelium of these fungi. MAUPAS ('79, p. 252) records having seen many nuclei in the hyphae of *Empusa muscae;* while VUILLEMIN ('86) published drawings showing a similar condition in *Entomophthora gloeospora* Vuil. FAIRCHILD ('97) also mentions having noted the multinucleate mycelium in certain species of Empusa. BREFELD, who has studied the group

more than any other investigator, has noted also ('84, p. 41) that the hyphae of Conidiobolus, which grows parasitically on Exidia and similar fungi contain many nuclei; but he contributes no comment on the internal structure of Empusa, whose external characters and development he has described in great detail.

CAVARA next published ('99) some cytological observations on *Empusa muscae*, which was shown to have multinucleate conidia, and on *Entomophthora Delpiniana*, with multinucleate conidia; and he showed the importance of this character in delimiting the groups of the Entomophthoreae, a point with which I heartily agree. But CAVARA'S account of the division of the nuclei in these two forms by simple fragmentation is without doubt incorrect, as is plain from the complicated method described in the present paper.

Finally, GALLAUD (:05) has quite recently studied a form, Delacroixia, apparently similar in habit to Conidiobolus, whose mycelium as well as conidia contain numerous small nuclei.

The application of refined technique to the study of the cytology of these organisms has resulted in but one paper—that by FAIRCHILD ('97) on Basidiobolus—which deals exhaustively with the nuclear details. EIDAM ('86), who first discovered Basidiobolus and figured its uninucleate cells, and LOEWENTHAL (:03), both working with unsectioned material, and quite recently WOYCICKI (:04), have also contributed certain cytological observations in their studies on this form.

Basidiobolus shows, as we shall see, little resemblance cytologically to Empusa, and RACIBORSKI ('96) contends that it should not be included in the Entomophthoreae. However, since this form is generally considered in connection with the group, it seems best to review at this time FAIRCHILD'S account of the nuclear division in Basidiobolus. This author has described in great detail the peculiar division by which the two small beak-cells are cut off from the adjoining gametes. The division of the nuclei in these beaks bears little resemblance to that in Empusa, nor, indeed, to the process in any other thallophyte so far described; it rather resembles, according to FAIRCHILD, that in higher plants, in that a cell-wall is laid down through the instrumentality of a cell-plate. During the prophases of division, the nucleole disappears, and the author thinks it is probably used to form spindle

fibers. The nuclear membrane, as in the higher plants, appears to be dissolved and a barrel-shaped or cylindrical multipolar spindle is formed. Strongly staining granules terminate each of the poles of the broad spindle, and in the early phases, the many chromosomes gather in an equatorial plate. In the anaphases, a double row of granules appears in the equator of the spindle, which is regarded by the author as forming a true cell-plate, since the new cell-wall is laid down between them. It should be noted in this connection, however, that such a cell-plate appears to lack the earlier fusion of the fibers, which, in the higher plants, invariably precedes the splitting and the subsequent deposition of cell-wall substance between the two new plasma membranes thus formed. Vegetative nuclear division was also observed by FAIRCHILD, who evidently regards the process as essentially similar to that just described, although in this instance he did not succeed in finding a cell-plate.

WOYCICKI (:**04**), on the other hand, while agreeing with FAIRCHILD in general as to the events of mitotic division in Basidibolus, confirms RACIBORSKI'S assertion (**'99**) that the new cell-wall grows centripetally as a ring-formed growth, like that in Spirogyra, and in this case entirely independently of the spindle.

NUCLEAR DIVISION.

The nuclei in the coenocytic hyphae of Empusa are comparatively large, measuring frequently as much as 7–9 μ in diameter, and are thus especially favorable for a study of the phenomena of nuclear division. In the vegetative hyphae they are usually spherical when in a resting condition; while in conidiophores or in similar elongated cells the nuclei also often become greatly elongated. In the conidiophores of *Empusa* sp. (*figs. 23, 25*[2]), the resting nuclei may even assume irregular and apparently amoeboid shapes.

The resting nuclei of the vegetative hyphae of *Empusa sciarae* (*figs. 19, 27, 57*) have no nucleole-like bodies whatever, whereas in other forms, e. g., *E. muscae* (*figs. 38–40*) and *E. culicis* (*fig. 48*), each nucleus possesses one sharply defined nucleole. In still others, *E. aphidis* (*fig. 44*) and *Empusa* sp. (*figs. 22–26*), the number of nucleoles

[2]Figures numbered 1–48 will be found on *plates XIV* and *XV*. *Figs. 49–67* are on *pl. XVI*, herewith.

varies, since one, two, or sometimes even four such bodies may be normally present in a resting condition. In many cases where these structures do occur, they appear to be surrounded by a clear space, and some show a filamentous connection with the chromatin (*figs. 22, 44*). In other instances (*figs. 47, 48*), no such clear space is seen, but the nucleole appears instead to be closely surrounded by a mass of chromatin.

In optical sections, the nuclei of *E. sciarae* (*fig. 57*) show darker granules which are apparently connected by more lightly staining portions, thus giving an appearance corresponding to the common conception of the chromatin and linin in the resting condition. But careful focusing reveals rather a more or less homogeneous, much convoluted thread, or filamentous material. Since I cannot, in fact, see any appreciable differentiation into chromatic and achromatic portions, I am inclined to regard the chromatin in this instance as resting in the form of a spirem thread.

I am hardly prepared, however, to accept for these nuclei the ideas of VAN WISSELINGH ('99) and of GRÉGOIRE and WYGAERTS ('03), who think that there is no distinction between linin and chromatin. For, though it is true that in the resting nuclei of Empusa the nuclear material appears to be homogenous, during mitosis, on the other hand, some parts retain the stain much more tenaciously than other parts. One may bleach out an iron haematoxylin preparation, for example, until only that portion of the dividing nucleus immediately around the centers remains dark. However, whether this difference brought out by staining is due to mere physical causes, I cannot say.

Resting nuclei take the stain readily and are thus sharply differentiated; whereas those which are in a state of division stain less deeply. Hence in searching for division-stages, one has but to find those nuclei which are lightly stained and from which the color has been more washed out. But this applies apparently only to those nuclei which are somewhat advanced in the process, for such differentiation is not readily noticeable in very young stages. The earliest stages of nuclear division in the two species of Empusa in which I have studied the phenomenon, *E. sciarae* and *E. aphidis*, in fact are not altogether clear. It is to be hoped that other species will prove more favorable for the beginnings of the process. It is not quite clear, for example,

just what events are transpiring in such a nucleus as that figured in *fig. 49*. But from the later condition shown in *fig. 50* to near the close of the telophases, a great abundance of successive stages affords an easy interpretation of most of the events of nuclear division.

It is highly probable that *fig. 49* illustrates the early divergence of the two centers of division present in the middle of the nucleus, although no clew is given in the preparation toward the solution of the puzzling question as to the origin of these centers. In this figure a clearly defined strand connects two darker regions, where, presumably, nuclear material is being aggregated. A clear space, probably a cavity filled with nuclear sap, separates the two centers and encloses the connecting filament. Between *fig. 49* and *fig. 50* is plainly a large gap. In the latter, the two centers are large, conspicuous, intranuclear bodies, from each of which radiate in all directions granular fibers. These fibers appear to connect in some instances midway between the centers with those from the opposite system of fibers; others appear to cross over the equatorial region and to be independent of the other system.

Figs. 51–61 record successively the phenomena attending further divergence of the centers and the massing about them of the material of the divided daughter-halves of the nucleus. It may be noted in these preparations that one of the first evidences of the activity in a nucleus leading to division is the change from a globular to an oval form. During the progress of the internal mitotic changes, the nucleus finally becomes elliptical or oblong and greatly elongated. It may readily be noted also that the long axis of the dividing nucleus corresponds generally with the long axis of the filament. In some instances, however, the nucleus lies obliquely across the hypha, presumably carried about by cyclosis.

An increasing abundance of nuclear sap is shown in *figs. 49–58*. In *fig. 49*, the clear portion is seen to occupy the space between the two diverging centers. From the repeated occurrence of similar nuclei in which the middle appears to be occupied by a clear space, it seems probable that one of the earliest manifestations of mitotic activity in the case of Empusa is the accumulation of karyolymph in the immediate vicinity of the intranuclear centrosomes. In *fig. 50* the nuclear sap apparently lies both between the two centers and in the interstices

between the fibers. In *fig. 51* a clear space is noticed at one side of the dividing nuclear elements, whereas, in the more advanced stages shown in *figs. 52–55*, sap lies mainly between and separating the two active centers of division. In *figs. 54* and *57*, a region almost free from fibrous material separates the daughter halves and gives the appearance of a turgid, intranuclear, vacuolar cavity. It will be noted in these instances as well as in still later stages (*figs. 56, 58, 60*), that this nuclear fluid appears to exert pressure on the chromatic elements, as evidenced by the curved line where the massed chromatic material borders on the vacuolar fluid. In *fig. 56*, which shows the next step in the division of the nucleus following *fig. 57*, the cytoplasm has constricted in two the mother-cavity, and in this figure as well as in the similar stages shown in *figs. 58, 60*, it will be at once noticed that the solid constituents of the young daughter nuclei occupy a pseudosynaptic position, and that the greater part of the cavity of the daughter nucleus is occupied by a clear space. Whether an osmotic pressure of the intranuclear fluid causes this appearance, or whether it is due simply to the massing or contraction within the nuclear cavity of the chromatic elements about the polar centrosomes, can hardly be determined with certainty, but it is probable that both forces are thus operative.

Fig. 58 illustrates an interesting deviation from the more common median constriction of the elongated nucleus shown in *figs. 56, 59, 60*. Here a double cytoplasmic constriction has taken place, resulting in two daughter nuclei and between them a vacuole, which is undoubtedly filled with sap from the mother-nucleus. Probably in this instance the dividing nucleus became so greatly elongated that surface tension operated in such a way that the encroaching cytoplasm constricted it into three parts instead of two, as is usual.

In *fig. 59* is shown an early telophase condition in which the solid constituents of the nucleus are being redistributed throughout the daughter nuclei. Here the movement of chromatic material, as is characteristic for nuclei in this condition, is opposite to that seen in early stages of nuclear division. Whereas in the early phases this material moves toward and masses about the polar center, in the teleophases, it moves in the opposite direction, away from the center. In *fig. 59* the center in each nucleus is still conspicuous, although a

considerable portion of the mass, especially that in the upper daughter nucleus, has moved centrifugally, towards the nuclear membrane. We note also in the upper nucleus of this figure what appears to be a thickening at the outer ends of the radiating filaments, and in the lower nucleus some of the radiations are seen to be double.

I am inclined to interpret *fig. 61* as a very late telophase, and as a near approach to a resting nucleus; between this figure, however, and *fig. 59* there is obviously a wide gap. Such nuclei as that shown in *fig. 61* are comparatively common, however, and without doubt represent a stage in which the center now exists only as a focal region for the attachment of the chromatic fibers to the nuclear membrane. Occasionally one may see at this focal point, especially in preparations stained with the triple stain, a very dimly defined body, apparently a remnant of the old center, lying against the nuclear membrane. But in similar preparations stained with iron haematoxylin, the core of the old center seems to be entirely empty, while immediately around it a dense chromatic mass persists for some time. In *fig. 61*, for example, there remains hardly any visible evidence of the old center of division; a few conspicuous fibers and a darkly stained mass which was accumulated about the center remain, however, to mark its former position, and the fibers now serve apparently to attach the main mass of chromatin to the nuclear membrane.

Apparently such a nucleus is "polarized," at least in so far as there seems to be a special and possibly permanent focal point on the nuclear membrane for the chromatic materials. Whether this is centralized in the same sense as Euglena, or permanently polarized, as in the case of Phyllactinia (HARPER, :05), must be settled by further investigation.

We see that, from very early stages, the centrosomes in these dividing nuclei are conspicuous bodies, which grow larger and more conspicuous as division progresses, and this is due, in my opinion, to the accumulation of nuclear materials about them. Each centrosome is lighter in the middle and has a darker rim (*figs. 50–67*), a phenomenon which I am convinced is partially due to refraction. But careful washing out of the stain sometimes leaves the middle totally bleached out, while immediately around it some parts retain the stain. Each centrosome thus appears possibly to have a core of plastin and a rim of chro-

matin, as is claimed by SCHAUDINN for the similar bodies of Coccidium. *Fig. 61* could therefore be interpreted as showing the rim of chromatin, but the plastin substance of the middle core has entirely disappeared.

The division of the nucleus just described for *Empusa sciarae* takes place in the later vegetative stages, when cross partitions are frequent and when the coenocytic cells are consequently comparatively short. Among the four or five nuclei present in each cell in this condition, we may occasionally find two nuclei in a state of division; generally but one, however, divides at a time. The nuclei in a certain cell do not, therefore, divide simultaneously, but each appears to act in entire independence of neighboring nuclei.

There occurs in earlier stages of the vegetation of the fungus an interesting modification of the process as described above. *Figs. 62–65* illustrate late stages in the division of the nuclei found in long coenocytic cells, in which cross-partitions are few and far apart. It will be remembered that during the earlier vegetative activities of *Empusa sciarae*, nuclear division takes place much more rapidly than cell-division, with the result that septa occur at rare intervals, while, on the other hand, nuclei during this period are abundant. When we come to compare the seemingly different type of nuclear division shown in *figs. 62, 63* with that shown in *figs. 50–55*, we note in each the intranuclear centers and the radiating chromatic filaments mentioned above. But here in the latter type the dividing nuclei assume an hour-glass shape, similar to those of Coccidium as shown in certain of SCHAUDINN'S drawings, instead of the oval or elliptical shape characteristic of the nuclei during the division above described. A careful comparison, however, leads to the conclusion that the only essential difference between the two types of division is in the amount of nuclear sap. In the latter case there is lacking the clear space filled with nuclear sap, between the separating chromatic filaments, so conspicuous in the type above described; or at least the fluid is much diminished in quantity. In *fig. 65* some is still present in the constricted region; but between the separating daughter halves in *figs. 62, 63*, as well as in *fig. 64*, little sap, if any, is evident. *Fig. 65* shows, in fact, a transition between the elongated, hour-glass shaped nuclei of the latter type and the oval ones of the former.

There can hardly be any doubt, especially after we make comparative observations on *Empusa aphidis*, which has a similar type of nuclear division, that such stretched-out nuclei as are shown in these figures get their peculiar form from the currents of protoplasm flowing in these long coenocytic hyphae. Resting nuclei, as is well known, are plastic to a remarkable degree, and thus, in long cells, may frequently become much elongated; so it seems more than probable that these dividing nuclei may likewise become stretched out in the same way.

Figs. 66, 67 represent poorly stained nuclei of *Empusa aphidis* in which division is taking place in a manner evidently similar in every respect to that described above as the second type. Here too we have vegetative hyphae in which septa are few and far apart; hence the general protoplasmic movements must disturb considerably the dividing nuclei. Practically all of the nuclei of this species conform to the type shown in *figs. 66, 67*, for I have but once found a doubtfully elliptical nucleus. The fact that the second type of division alone occurs in the long tubular filaments of *Empusa aphidis* points therefore to the conclusion that the stretching out of the dividing nuclei in these instances is brought about by cyclosis. In this second type of division here described, we can readily imagine that the protoplasmic currents also assist materially in the constriction and final separation also of the halves of the dividing nucleus. We may thus conceive, in the one case, of the protoplasm as undergoing such limited movements on account of its confinement in a short cell, so that the dividing nucleus is but little disturbed, and consequently, by the accumulation of karyolymph, it assumes a short oval or rounded shape; whereas in instances where the cells are long and the protoplasmic currents therefore stronger, the dividing nuclei become drawn out and elongated, and constriction becomes very early evident.

In *figs. 64, 65*, we note an interesting phenomenon. Here occurs an infolding at the poles, giving an appearance as if some stress had indented the nuclear membrane at this point. I have not observed this phenomenon in the oval nuclei of the first type, but it apparently occurs not infrequently in nuclei of the second type. It is possible of course, that the infolding may be an artifact, caused in some manner by the reagents. Such cases furnish indisputable proof, at any

rate, that the intranuclear centers are strongly anchored to that nearest portion of the nuclear membrane situated poleward from them.

We may summarize these results pertaining to the nuclear division of *Empusa sciarae* and *E. aphidis* as follows. During the early stages of division the nuclei become less stainable and slowly change from a rounded to an oval shape. Two diverging centers of division, or centrosomes, become conspicuous near the middle of the nucleus. Fibers may now be seen radiating from the two intranuclear centers, some crossing the median line between the centers, others evidently anastomosing with fibers from the other system. The nucleus elongates still more and the opposed centers, each with its system of radiating fibers, diverge farther and farther apart. The centrosomes appear to increase in size as division proceeds, probably from the aggregation about them of the chromatic material in the radiating fibers.

In cells which are comparatively short, a space filled with sap is early apparent between the diverging daughter masses, as well as in the interstices between the chromatic fibers. This sap increases in amount until in the oval, turgescent nuclei found in such short cells, the middle portion becomes filled with it, and we note a clearer central part, containing at first a few scattered fibers, separating the two polar, darkly-staining regions. On the final withdrawal of the last chromatic filaments to the daughter-poles, the middle of the elongated nucleus becomes perfectly clear and transparent. The cytoplasm now encroaches on the median sap-cavity and, by constriction, cuts the mother-nucleus in two. In some instances, a double cytoplasmic constriction may take place, so that a vacuole filled with nuclear sap is cut off and left between the two daughter-nuclei.

In long cells, on the other hand, or in filaments with few, far-separated septa, the nuclear sap does not accumulate in the manner just described; hence the nucleus, instead of becoming turgid with the liquid secretion, becomes early in the process of division constricted in the middle and greatly elongated, thus assuming the shape of an hour-glass. A few connecting strands in the constricted portions remain for some time, while the active polar regions, with their dense accumulation of chromatic material, become separated farther and farther, with the result that the two daughter-halves are finally pulled apart.

The lack of accumulation of nuclear sap in the latter type of nuclear division constitutes the only difference between this type and the one described above.

The accumulation of sap in the nucleus in the first instance is probably due to the lack of disturbance of the process by the restricted protoplasmic currents in the short cells. The lack of accumulation of sap in the second instance is probably due to the disturbing influences of the stronger protoplasmic movements which undoubtedly take place in the long tubular filaments. In the first type the chromatic substance in the newly formed daughter-nuclei comes to lie in a mass at one side of the nuclear cavity, thus resembling somewhat a synaptic condition. In the other, the nuclear materials of the young daughter-nucleus, massed about the centrosome, are closely enveloped by the surrounding cytoplasm, and not until later in the reconstructive processes which follow, does the nuclear sap appear.

Towards the close of division, the center in each nucleus comes to lie close to, if not actually on, that portion of the nuclear membrane nearest the pole. Its attachment and anchorage to the nuclear membrane is proven by the frequent indentation of the membrane at this point. In the young nucleus the center remains conspicuous for some time, but finally, with the resumption of a resting condition, it becomes entirely lost to view. In the resting nucleus, the nuclear materials appear to be distributed more or less evenly on a much convoluted, seemingly homogeneous, filamentous thread which resembles a spirem.

We have now to emphasize, before entering upon a discussion of the general bearing of these facts, certain peculiarities at once noticeable in this primitive mode of nuclear division. In the type first described, the nuclear membrane plainly persists throughout the whole process of division; also in certain nuclei of the elongated type, it undoubtedly persists (*figs. 65–67*), although it is here not so conspicuous. I am inclined further to regard a membrane as present around the chromatic fibers in *figs. 62–64*, notwithstanding the fact that in the preparations it cannot be seen. The iron haematoxylin stain is probably accountable for the failure to bring out the membrane clearly in this instance. Hence we may record at this point that in the case of Empusa, the nuclear membrane is at least usually persistent through-

out the whole of nuclear division, and that, consequently, the entire process is intranuclear.

Secondly, we note the absence of any definite chromosomes in this peculiar division; and equally noticeable is the failure of the chromatic material to become aggregated into an equatorial plate, as well the want of a definite achromatic spindle. Careful counts, however, of the fibrous strands radiating from the centrosomes indicate the probability of a constant number of these chromatic fibers. I have in many instances counted about sixteen of these radiations from the polar view (*fig. 55*), but it is perhaps impossible to determine exactly the correct number, on account of the great confusion of threads. I believe, nevertheless, that these fibrous strands of chromatic material represent the chromosomes, and further, that the two daughter nuclei each receive an equal number.

There seems little evidence for the existence of a differentiated achromatic spindle, but further study in related species may possibly assist in determining what here may correspond to such a structure. It is true that in *fig. 64* is shown an indefinite, intrafibrillar substance which might be taken for a spindle, but I am convinced that the thickness of the section in this instance is responsible for this misleading appearance. Careful observation reveals chromatic fibers in a lower plane of focus and it is their great number and close proximity in the background that probably causes the indefinite, washed-out appearance between the sharply defined filaments. In *fig. 54* also there is shown a similar substance between the radiating fibers, whereas in *fig. 55* this is not so noticeable. *Fig. 50* as well shows but little nuclear substance other than that in the sharply defined chromatic fibers radiating from the two centrosomes.

Since all the dividing nuclear substance outside the centers is apparently confined to the two systems of filamentous structures radiating from the centers, we must therefore conclude that there is no intrafibrillar spindle-substance. And, since we see also that these radiating strands appear to be chromatic in their staining reactions and not achromatic, the only conclusion which seems possible is that there is no substance in the dividing nuclei of Empusa which can correspond to an achromatic spindle. I am not prepared, however, for such an extreme belief, which would obviously much belittle the

importance of a fibrous mechanism for the accomplishment of mitotic division.

I should prefer to believe that the achromatic spindle substance, probably present only in small amount, is a part of, and inseparable from the deeply staining radiations. Should this be true, then we may conclude that the kinoplasmic spindle-mechanism is bound up closely with the radiating parts corresponding to the chromosomes. Possibly the chromatin is here more nearly a liquid substance than is usual, hence it may diffuse more readily throughout the linin basis, so as to be indistinguishable from the latter. At any rate, I should regard the chromatic filaments radiating from the centrosomes as corresponding in part to the fibers of the more differentiated spindle of higher organisms; and, further, since these mark the paths of the chromatin, they must also correspond to the mantle fibres. In the case of Empusa, so far as studied, there is obviously nothing which can correspond to the central spindle of more complicated nuclei.

CELL-DIVISION.

Cell-division in Empusa, as in many other lower plants, takes place in entire independence of nuclear division, and also apparently remote from nuclear control. There is concerned in the process no such fibrous structure as a cell-plate; since, in fact, no cell-plate is ever formed at the close of the nuclear division described above. Further, cell-division may not take place till long after all division of the nuclei has ceased; hence coenocytic hyphae result.

The branched conidiophores of *E. sciarae* (*figs. 16, 18*), as well as conidia in the process of abstriction (*figs. 28, 30, 31, 36*) furnish especially fine material for the study of cell-division. Examples are also occasionally met with in sections of vegetative hyphae (*figs. 19, 21*). A striking feature of the process as seen in conidiophores and young vegetative hyphae is the fact that in the cleavage of the cell, the new ring-formed partition-wall grows across a wide vacuolar space. In the case of the abstriction of the conidia, on the other hand, and probably as well in older vegetative stages, although I have not as yet seen the phenomenon in the latter instance, the new wall grows through a mass of cytoplasm. *Fig. 18* shows clearly the method of growth progressively inward of the ring-formed septum.

The plasma-membrane which bounds externally the thin primordial utricle has evidently been infolded at this point, thus forming a deep, narrow furrow. The young partition-wall which is being deposited in this groove can not be seen in the figure. We note further in *fig. 18* that the two nuclei which are shown are in a state of rest; in fact, nuclear division does not occur at all during the pre-fructifying period characterized by the formation of conidiophores. And in the same figure we also see that the nuclei are separated by a wide vacuolar space from that part of the cell in which division is proceeding, and that they are joined to the active region only by a narrow cytoplasmic connection. It seems reasonable to suppose that cell-division, in this instance, is a cytoplasmic phenomenon and is merely remotely or indirectly subject to nuclear control. In *fig. 18* it will be noted that the stain is deepest at the inner margin of the cleft, showing that in this innermost region in which the new wall is being laid down, the cytoplasm is densest and most active.

Fig. 19 shows a similar ring-formed septum partly across a young vegetative hypha, at a slightly advanced stage of growth. A bridge of cytoplasm is next thrown across the vacuolar space before the wall is completely formed, as is seen in *fig. 20*. This figure brings out most clearly the region of greatest activity. In the preparation, the stain (iron haematoxylin) was well washed out, so that the cytoplasmic bridge as well as the ring-formed wall are left unstained except at the innermost part of the furrow, where a small black granule is conspicuous. In this dark region the new wall is evidently being deposited. Immediately on the throwing across of the cytoplasmic bridge, the greater turgor of the cell below ordinarily causes the partition to bend outward toward the outer end of the hypha (*fig. 20*). This bending is also quite noticeable after the final completion of the partition wall (*fig. 17*).

A study of these figures might lead to the conclusion that we have here a process exactly similar to that already described for certain other fungi (see HARPER, '99, p. 506), in which the cleavage furrow first cuts across the cell and the wall follows later. One would in fact naturally come to this erroneous conclusion, since every one of the drawings mentioned above, except perhaps *figs. 19, 21*, shows clearly the circular furrow, but no sign as yet of the ring-formed septum.

In these preparations, however, the thin, delicate walls are not at all easy to differentiate. I am convinced that, unlike the cases just referred to, in Empusa a delicate wall grows simultaneously with the cleavage-furrow and not later. The figures which show abstriction of the conidia furnish sufficient evidence for this conclusion. In this case, the process of abstriction takes place essentially like the cell-division described for conidiophores, except that here the cleavage-furrow grows through a mass of cytoplasm instead of through a central vacuolar space. In *fig. 38*, the completed wall separating the conidium from the basal cell of the conidiophore may be plainly seen, since the protoplasm is shrunken away on both sides. But in *figs. 28, 30, 31, 36*, although the cleft itself at the base of the conidium is brought out with diagrammatic clearness, the wall which accompanies it is not so evident. Two reasons may be noted here, however, which are not so apparent in the case of conidiophores, for the conviction that the partition-wall is also present in these instances. The wall which cuts off the conidium, when completed, as was noted above in the case of the newly formed septa in conidiophores, is forced upward by the greater turgor of the basal cell, and here forms a kind of columella within the conidium. While it is possible that the cleavage-furrow itself might be stretched and forced upward in this fashion, yet it is more than likely that the unsupported plasma-membranes bounding the cleft could not withstand the considerable pressure which is developed. A further reason for the belief in the necessity of the cooperation of a ring-formed wall in these instances is seen in the shooting off of the conidia immediately on the completion of their abjunction. In *figs. 29, 37, 43*, are shown conidiospores which have evidently just been shot off and in which the turgescence of the protoplasm has now reversed the position of the cross-wall, making a papilla at the base instead of an indentation. We see clearly in *fig. 37* the delicate wall shrunken away from the spore-plasm. An uncompleted wall at the time of the discharge of the conidium would evidently allow the escape of the protoplasmic contents.

GENERAL DISCUSSION.

It is clear that the division of the nuclei of Empusa which has just been described, although apparently resembling in some respects

amitosis, is certainly much more complicated than a mere mass division such as occurs in the latter process. In the division of the centronucleus of Empusa, we have, as was seen, intranuclear centers of division, or centrosomes, which function as active agents in nuclear division. Centrosomes, when they do occur, are, on the other hand, supposed to take no essential part in amitotic division. In the dividing nuclei of Empusa, we have also, besides active centrosomes, an arrangement of the chromatin in radiating fibers comparable to chromosomes, and, further, a simple spindle-apparaatus. I should therefore separate the process in this form far from amitotic division, although still regarding it as an extremely simple type of mitosis.

In the division of the nucleus in Euglena, the resemblance of the phenomena to amitosis was regarded by KEUTEN as so striking that he called the process in this organism a simple intergradation between direct and indirect division. In the case of Coccidium SCHAUDINN remarks that the division of the nuclei takes place by a "primitive mitosis." Should SCHAUDINN be able to find, further, as is probably possible with improved fixation, the division-centers in his second kind of division, which occurs in the stages following the fertilization of the egg, he should come to the conclusion that he has here also not, as he concludes in his paper, a still simpler type than the first, but a primitive mitosis essentially like the first. For in the event of similar intranuclear division-centers occurring in both cases, he would have two types of division somewhat comparable to the two types mentioned above in Empusa, which, as we have seen, differ from each other only in the amount of nuclear sap present, and in the earlier constriction and elongation of the second type.

In Empusa, Coccidium, and Amoeba, the absence of an arrangement of the chromatin during the prophases of nuclear division in an equatorial plate, attests the extreme simplicity of the mitotic process in these instances. The absence of this equatorial arrangement leaves us, in fact, unfortunately in doubt as to the manner of the equal distribution of the chromatin between the two daughter nuclei. If we accept, however, the commonly accepted doctrine that "the daughter nuclei receive precisely equivalent portions of chromatin from the mother nucleus" (WILSON, :00, p. 70), we must conclude

that this equal division of the chromatin occurs somewhere in the obscure prophases; in Empusa, e. g., probably long before the appearance of the conspicuous centers seen in *fig. 50*.

The absence of the arrangement of the chromatin into an equatorial plate prior to the divergence of the two daughter masses possibly results from the poor development of the achromatic spindle, due to the small amount of linin present in the nucleus. To this same cause is probably due also the failure to form definite chromosomes in these simple organisms. In Amoeba, according to SCHAUDINN'S observations ('**94**), there are apparently no radially arranged chromatic filaments; while in Coccidium (SCHAUDINN, :**00**) and Empusa, evidently a still higher type obtains, since in both these instances we have formed, rather late in division, filaments of chromatin, which undoubtedly correspond to the chromosomes, and are radially arranged about the centrosomes.

The formation of an "equatorial ring" in the nuclear division in Euglena, and of a more compact equatorial arrangement of the chromatin in Euglypha (SCHEWIAKOFF, '**88**), Actinosphærium (HERTWIG, '**98**), Paramœcium (HERTWIG '**95**), Aulocantha (BORGERT, :**00**), and other Protozoa, certainly indicates the presence in these forms of a more highly differentiated mechanism for the halving of the chromatin. In all these cases, we note the early formation of chromosomes, which are usually very clearly defined, and generally a well developed spindle, consisting of both central spindle as well as polar mantle-fibers; so that we are justified in the conclusion that in these more highly differentiated figures there is a greater amount of intranuclear achromatic substance present than in the nuclei of Empusa and Coccidium.

We may compare at this point the degree of differentiation of the intranuclear spindle in these organisms. KEUTEN regards the dumbbell shaped nucleolo-centrosome in Euglena as probably serving as a spindle mechanism; and BOVERI (:**00**) and CALKINS (:**01**, :**03**) also think that the strand of connecting substance in this constricted nuclear body corresponds to the central spindle of higher organisms. CALKINS (:**01**, p. 265) points out in this connection that Paramoecium furnishes a clew to the relationship of such connecting strands in Euglena to the fibrillated central spindle, since in Paramoecium the

"central portion of the division-figure is a single strand which widens and becomes fibrillated at the ends." SCHAUDINN (:00, p. 229) evidently does not so regard the corresponding portion of the dividing nucleus in Coccidium, since he calls this connecting strand simply "Verbindungsfaden der Tochterkarysome," and says that "von Spindlefasern und Poldifferenzirungen ist keine Spur wahrzunehmen." I am also inclined to believe that no part of the constricted nucleolar body in Euglena and Coccidium is homologous with the central spindle of more complicated nuclei, since in all cases where a structure occurs which can be positively referred to the central spindle, it consists of usually distinct fibers which extend between and connect the diverging chromosomes. In these instances, the connecting portion of the dividing nucleolar body bears no such relation to the chromatic filaments, but instead it lies simply as a slender core in the axis of the mitotic figure. Further, in the centronucleus of Empusa, which is undoubtedly similar in every respect except this one to that of Coccidium, such a connecting body does not occur at all, unless, indeed, it be represented in *fig. 49*. Therefore, the strand connecting the constricted nucleolo-centrosome of Euglena and Coccidium, in my opinion, does not represent, phylogenetically, the central spindle, nor in fact any structure of the higher nuclei, but is a structure which is confined, so far as yet known, to these two Protozoa. It is just what SCHAUDINN calls it, viz., simply a drawn-out filament connecting the daughter centrosomes, which has no apparent function. On the other hand Paramoecium, as shown in HERTWIG'S figures, shows a true central spindle, and the final median constriction of this spindle and the consequent aggregation of the fibers of the middle portion into what appears to be a single strand, does not present a figure which can be in the least compared, as CALKINS claims, with the nucleolo-centrosome described above. If there be any indication at all of central spindle in these simpler centronuclei, then, in my opinion, it must be looked for in the dimly defined, continuous, bluish substance, for example, shown in the drawings of Coccidium (see SCHAUDINN'S *figs. 31, 32*), which lies between the daughter chromatin masses. SCHAUDINN himself, however, evidently believes that these are not spindle fibers. In the case of Euglena, the central spindle is probably represented by the dim

achromatic substance remaining between the separated chromosomes, e. g., in KEUTEN'S *fig. 11*. But in Empusa, there is no appreciable achromatic substance in the corresponding equatorial region of *figs. 62, 67*. There is, therefore, according to my interpretation, in the simple cases where no equatorial arrangement of the chromatin takes place, practically no development of a central spindle; but whether these two facts are related somehow as cause and effect must await further investigation. Hence we may regard the intranuclear figure in the case of Empusa and Coccidium, as an extremely simple apparatus, which consists merely of the two opposed centers of division, each with its system of polar radiations. Further, these polar rays must all correspond in function to the mantle-fibers, instead of in part to the extranuclear polar asters of the higher animals, since they all mark the paths of movement of chromatin material. As seen in *figs. 50, 54, 55, 59*, for example, the fact is quite apparent that the intranuculear centrosomes lie some distance from the nuclear membrane, and that there is no appreciable differentiation in the radiations which extend in all directions from them. All appear alike to consist, at least in part, of chromatin material. In later stages, represented in *figs. 64, 65*, the centers appear to have been pulled to the periphery so that they come to lie against the nuclear membrane. I am inclined to think that this peripheral position represents the ultimate fate of all of the centrosomes, since the very last stages (e. g., *fig. 61*) almost invariably show the old centers lying at one side against the nuclear membrane. Such figures lead us to believe that after all there may be a slight differentiation in the astral radiations, since those fibers which attach the centrosome to the nuclear membrane may be mainly concerned in this peripheral movement of the centers, forming in these instances a sort of "antipodal cone" of fibers. At any rate, while there may be, in such a spindle, certain polar structures which appear to have a special function and thus to form an "antipodal cone," there is no such striking differentiation of the aster into a "principal cone" and "polar rays" as was described by VAN BENEDEN.

In those more complicated centronuclei in which the chromatin is gathered during nuclear division into an equatorial plate and in which definite chromosomes are formed, as in Euglypha, Paramoecium, and

other Protozoa, a more or less clearly defined, fibrous, central spindle is found in addition to the mantle fibers. The absence of the central spindle in the simpler type of intranuclear division seen in Amoeba, Coccidium, and Empusa, and its meager development even in more complicated cases, clearly suggest that the central spindle-fibers, when present, play only a minor rôle in nuclear division as maintained by HERMANN ('91), viz., that they are non-contractile supporting elements, which form a basis on which the movements of the chromosomes take place. The chromatic structures in Empusa are undoubtedly moved poleward without the assistance of such connecting fibers, and they seem to be supported entirely by the surrounding nuclear sap.

These facts may be interpreted as thus furnishing a strong argument against the acceptance of the "pushing theory" of DRÜNER ('95), who supposes an active growth or elongation of the central spindle, thus pushing the spindle-poles farther and farther apart; and at least in part against the suggestion of MOTTIER (:03, :04), who thinks that the chromosomes may be conveyed to the poles both by a pushing and a pulling action of the spindle-fibers.

No clear explanation of the mechanism which accomplishes these primitive divisions has yet been marked out. As pointed out above, there are in Empusa no specially differentiated mantle-fibers, since the radiating astral rays of the intranuclear figure themselves mark the paths of the chromatin-movement. Whether the movements which take place in these radiations are similar to those which occur in the aster of the more highly differentiated extranuclear centrosphere, I cannot say, but this seems quite probable. In Empusa the radiations extend in all directions from the centrosome and some are anchored firmly to to the persistent nuclear membrane at its nearest point, while others project into the nuclear cavity, apparently ending free in the karyolymph. Now, a contraction of the radiating fibers would undoubtedly accomplish just the phenomenon which we see takes place. The fibers seem to shorten and to thicken, and an appearance suggesting an accumulation of darker staining material immediately around the centrosome results. The distal indentation of the nuclear membrane which we see occasionally (*figs. 64, 65*) should also be regarded as strong evidence that a pull of some sort or a contraction

of fibers in this region has taken place. But I can see no evidence in this instance of a using up of any of the material which has accumulated about the poles, as has been suggested by STRASBURGER (:00) to explain the shortening of the mantle-fibers in certain cases.

It may be pointed out in this connection that the fact that the fibrillar radiations in Empusa appear to be almost homogenous, and further, chromatic in their staining reactions instead of achromatic, does not seriously detract from the reasonableness of the contractile hypothesis, as applied to this form, since it is only necessary to assume that contractile linin is also present in small amount in the fibers, along with chromatin. WILSON ('95), in fact, maintains that in the case of echinoderm eggs, the fibers are derived not merely from the linin-substance, but also from the chromatin.

As in the telophases of mitotic processes in general, in the later stages in Empusa a centrifugal movement of the chromatin sets in, which may sometimes begin even before the two daughter-nuclei are separated by constriction from each other. *Fig. 59* shows such a late condition, in which the chromatin-movement seems to be of the nature of an active outward growth, since we now note at the distal ends of the fibers accumulations of darker and apparently denser material. Should we assume that the aggregation of chromatin about the centrosome in the first instance is brought about by the contractility of the kinoplasm in the radiations, then we must suppose that later some subtle change occurs in the body of the centrosome itself, or else in the fibrillar rays, to stop contraction and to set up an opposite growth of the fibers. But I should regard it as not an impossible assumption that the centrifugal movement in the latter instance might be brought about simply by a loosening up the chromatin in the increasing nuclear sap by which it is surrounded—a phenomenon which would probably follow as a mere mechanical consequence the final cessation of the forces which caused the centripetal movement.

The suggestion that these alternating centripetal and centrifugal movements of the chromatin are of the nature of flowing movements appears to gain some support in the case of Empusa. MONTGOMERY (:01, p. 352) concludes that this flowing movement of the nuclear materials is automatic; but I fail to see how this author can retain,

even in part as he does, the idea of the contractility of the secondary linin-fibrils, in addition to the above theory, since an automatic movement such as he conceives to take place should be regarded as an amoeboid movement in response to chemotropic stimuli. WILSON (:01, p. 575) also regards the chromatin as "a liquid substance which may be absorbed or given off by an achromatic basis such as plastin or linin, and may thus flow from one part of the nucleus to the other." The latter author appears to adopt to a certain extent the ideas of BÜTSCHLI ('92), in that in his studies on Toxopneustes he has become thoroughly convinced that the astral radiations are in part the result of centripetal currents, or diffusion-currents, of hyaloplasm converging on the centrosphere.

While it is quite possible that the chromatin in Empusa is a liquid substance which may flow or diffuse about through an achromatic linin basis, as WILSON suggests, this, in my opinion, does not preclude the idea of a contractile linin substance serving as the mechanism of mitotic division. I must say, however, that while entirely convincing evidence is lacking that the primitive mitosis in Empusa is accomplished by means of a contraction and a growth of the fibrillar, kinoplasmic radiations, there is, on the other hand, even less evidence in favor of other theories; for example, that the movement of the chromatin is automatic, due to chemotropic forces which are supposed to emanate from the centers; or that this movement is due to diffusion-currents induced by the chemism active at the centers; or that it results from magnetic or electrostatic forces, an idea which has been recently revived by LILLIE (:05).

In the primitive mitotic division characteristic of Empusa, we see but little resemblance to the corresponding process as described for other low plants. In all these cases, even in the Myxomycetes (HARPER, :00), a well-defined spindle and chromosome, and an equatorial arrangement of the chromatin may be observed. It is apparently very common among the thallophytes that the nuclear cavity and membrane persist during a large part of the mitotic processes; see, for example, figures of Erysiphe (HARPER, '97), of Albugo (STEVENS, :01), of Dictyota (MOTTIER, :00). But in all of those thallophytes in which centrosomes occur, the latter are *extra-* and not *intra*-nuclear bodies. Empusa is therefore in this respect unique

among the thallophytes and may be regarded as a primitive form; and further, the fact that it possesses intranuclear centers of division may perhaps be regarded as adding another point in favor of HERTWIG'S ('98) view as to the intranuclear origin (by the extrusion of centrosome) forming substances from the nucleus of the extranuclear centrosomes of the more highly differentiated organisms.

It has been already pointed out above that Basidiobolus, which has been generally regarded as a member of the Entomophthoreae, shows in its mitotic features (its broad, multipolar spindles, and its formation, according to FAIRCHILD ('97), of a cell-plate), as well as in other morphological aspects, wide differences from Empusa.

Cell-division by means of the growth inward of a ring-formed wall is apparently a common type of division among the filamentous thallophytes. Such a constriction of the cell has so far been shown for Beggiatoa (HINZE, :01), the blue-green algae, Ulothrix (DIPPEL '65), Spirogyra (STRASBURGER,'80), Cladophora (DAVIS,'04), the red algae, and a few other forms. WOYCICKI (:04), contrary to FAIRCHILD'S ('97) assertions, contends that the cell-wall in Basidiobolus also is a centripetal growth. The gametes of Sporodinia and the conidia of Erysiphe are cut off in a similar manner, except that, according to HARPER ('99), the ingrowth here is simply a deep narrow furrow and not the growth inward of a ring of fungus cellulose. The wall in this case is deposited later between the two plasma-membranes.

As has been shown in this study of Empusa, the ring-formed cleavage-furrow starts at a definite region of the plasma-membrane, sometimes remote from the nuclei; and further, the nuclei at no time appear to be concerned, directly, in the process. TOWNSEND'S ('97) observations on nucleated and enucleated fragments of protoplasm leave no doubt, however, as to the ultimate necessity of the presence of a nucleus, in order to initiate the cytoplasmic activities in Empusa which lead to cell-division. Whether the localized stimulus in this case results first in a deposit of a ring of cellulose-substance on the inner surface of the wall of the mother-cell, which might then by its growth progressively inward be regarded as the agent of cleavage; or whether there first occurs in this region an infolding of the plasma-membrane, thus resulting in a circular furrow, to be soon followed by a deposit of wall-substance in the cleft, I am not able to state. In either

case, at any rate, the importance of the plasma-membrane as a factor in such a cell-division should be emphasized. The process in Empusa, in fact, would seem to furnish an argument in favor of NOLL'S (:03) view that in Bryopsis the controlling factor of embryonic growth is located in the *Hautschicht*. Apparently in Empusa a definite region of the plasma-membrane is stimulated to action, a ring-formed infolding of the membrane occurs, and at once in the cleft thus produced, the new wall begins to be deposited. A darker accumulation, presumably of kinoplasm, may now be seen at the inner margin of the cleft (*figs. 18, 20*), where the activities leading to the cleavage of the in-pushing plasma-membrane and to the ingrowth of the partition-wall are evidently greatest. But the plasma-membrane does not alone seem to be the active agent for these phenomena, for *fig. 18* shows a darker portion, having appreciable thickness, which apparently marks a more or less broad region of concentration of kinoplasm. This fact, therefore, may be regarded as an argument against the plasma-membrane itself being the sole controlling factor in this case. Further, MOTTIER'S ('99) experiments on Spirogyra and Cladophora, in which, by reason of the disturbance due to centrifugal force, the cellulose-ring, when once begun, was never brought to completion, notwithstanding the fact that the plasma-membrane was still intact, furnishes very convincing evidence against the acceptance of the theory that the *Hautschicht* alone is the controlling factor of wall-formation in these instances.

The fact that the cleft and ring-formed wall are finally carried across a wide vacuolar space (*figs. 17–21*), will not permit of the application to this case of SWINGLE'S (:03) explanation for the mechanism of the cleavage in Rhizopus, Phycomyces, and other forms. For it seems impossible to conceive how local contractions of the cytoplasm could cause the constriction of the cell in the case of Empusa. We could perhaps think of such a contraction as initiating the process, but that these forces could obtain after the narrow diaphragm of cytoplasm had begun to be pushed across the central vacuole, seems to me inconceivable.

In certain instances in Empusa, as, for example, when a germ-tube is formed (*figs. 8, 9*), the end cell of a filament keeps cutting itself off from behind, thus enabling the body of the protoplasm to

travel forward, so to speak, and to seek a favorable environment in which to grow. BREFELD ('83) seems to think, in the case of the similar phenomenon in Ustilago, that the cells which are thus cut off behind are empty, and that in this way no protoplasm at all is wasted in the process. If this were true, a cell-wall would be formed from a single plasma-membrane, thus differing from the division described above, in which the membrane is split so that the wall is deposited between the two. But the apparently empty cell retains its turgescence for a time before collapsing, thus proving that there is at least a film of protoplasm present. Further, sections of similar conditions in which conidia are cut off from a basal shooting-cell (*figs. 28, 36, 38*), show clearly the thin primordial sheath of enucleate protoplasm in the lower cell. The fact that the protoplasm of these lower cells seems to undergo speedy degeneration contributes another point in favor of the idea of the vital importance of the nucleus in nutrition.

I am at a loss to understand why the conidium should be regarded by THAXTER ('88, p. 143) as a one-spored sporangium, since in all the sections of conidia which I have examined there is no sign of a second inner wall. It may be that the plasma-membrane of the plasmolyzed contents of a conidium may have been mistaken for a wall; or, again, it is possible that this author's *figs. 320, 321* represent conidia still surrounded by the slimy protoplasm which is sometimes discharged from the ruptured basal cell.

I wish in conclusion to express my hearty thanks to Professor R. A. HARPER for the privileges afforded in his laboratories; to Professor W. S. MARSHALL, for assistance in the determination of insects; and to the Carnegie Institution of Washington, for a research assistancy under which this work has been done.

SUMMARY.

1. *Life history.*—The life history of *Empusa sciarae* may be summarized as follows: The disease attacks both larvae and adults of the host, Sciara, causing ultimately their death. The young, uninucleate germ-tubes, after they have entered the body-cavity of the insect, grow there at the expense of the nutrient fluids. After the protoplasm has increased in amount, a branching, coenocytic myce-

lium is produced, which in early stages is few septate; later, however, at the culmination of vegetative activity, septa are abundant and branching becomes more frequent.

Finally, the body-cavity becomes almost completely filled with the mycelial filaments, vegetative activity ceases and the death of the insect ensues with the beginning of the fructifying condition.

Radial branches, which mark the beginnings of the conidiophores, are put forth from the short, 3–5-nucleate cells which make up the mature mycelium; in this species, but one branch grows from each cell. These radial hyphae bore their way out through the body-wall of the insect; some form the rhizoids which attach the host to the substratum, while others grow into branched conidiophores. Each conidiophore is cut up by cell-division into uninuclueate segments, each of which pushes out beyond the surface of the host and cuts off from its tip a single uninucleate conidium. The basal cell below the conidium comes to possess but a thin, enucleate primordial utricle, and it finally becomes greatly swollen from the absorption of water. Ultimately this swollen basal vesicle bursts in a ring at the top where it joins the conidial wall, or the columella-like wall may be split in some instances, and the conidium is thus shot violently away, the slimy protoplasmic contents of the lower cell being frequently carried along with the conidium and serving to stick the latter to the substratum. The partition which cuts off the conidium is at first curved upward by the greater turgescence of the vesicle; but when the spore is shot off, this reverses its former position, and in the conidium it appears as a prominent papilla.

2. *Nuclear division.*—The nuclei of Empusa are "centronuclei," since the centrosomes which are active during division are permanently intranuclear.

The division of the nuclei which takes place during the vegetative stages appears to be of the nature of a primitive mitosis, similar in many respects to that described for certain of the simpler Protozoa. The nuclear membrane generally persists during the whole process. A simple intranuclear figure is formed, which in later stages consists of the two opposed centers of division, to each of which converges from all sides a system of fibrous radiations. The many radiations which converge at the two poles correspond to the chromosomes; and,

although they appear to be chromatic in their staining reactions, they probably are made up principally of chromatin, and a small amount of linin. The chromatin at first concentrates about the centrosomes, which thus appear to have a darker rim about a lighter center. The centripetal movement, as well as the later centrifugal movement characteristic of the telophases, may be regarded as of the nature of the flowing or diffusion of a liquid chromatin through a contractile linin basis.

In the nuclear division of *Empusa sciarae*, the chromatin does not appear to pass through an equatorial plate stage.

We may distinguish two shapes of the dividing nuclei in Empusa: one found in short cells of the vegetative hyphae, in which the nuclei in the later stages of division assume an oval or ellipsoidal shape; and another found in long cells, in which the nuclei become themselves greatly elongated and early assume a constricted, hour-glass shape. In the oval nuclei, the nuclear sap accumulates so that the cavity becomes turgescent; while in the elongated nuclei, the liquid does not accumulate, at least not to such an extent as in the first instance, so that the consequent encroachment of the cytoplasm between the two daughter-halves results in an early constriction. In the long cells, cyclosis is doubtless stronger than in the short cells, thus bringing about in such instances a greater disturbance of the mitotic processes.

3. *Cell-division.*—Cell-division in *Empusa sciarae* is accomplished by means of the growth inward, from the wall of the mother-cell, of a ring-formed partition. In a majority of cases, the new cell-wall is carried across a wide, central, vacuolar space; when the older cells become filled with cytoplasm, however, and later when the conidium is abstricted, the wall cuts through the protoplasm which fills the cell. A ring-formed cleavage-furrow starts at a definite region of the plasma-membrane, and a wall is at once deposited in the cleft. A region of some thickness at the inner margin of the cleft, where the processes are most active which lead to the cleavage of the in-pushing plasma-membrane and to the deposition of the partition wall, stains darker than the surrounding cytoplasm. This fact is made the basis for the conclusion that the split plasma-membrane is not the sole active agent of cell-division, although it may be

a controlling factor in the process. Cell division, in this instance, is regarded as a cytoplasmic phenomenon, since the nuclei may be remote from the place of constriction; and, further, they appear to have nothing directly to do with the process. The ultimate necessity of the presence of a nucleus, probably as a controlling factor of nutrition, is proven, however, by the early death of the enucleate basal cells cut off from the conidia and from the end-cells of germ-tubes.

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EXPLANATION OF PLATE XVI.

The drawings were made with the aid of an Abbe camera lucida, together with the Zeiss 2 mm apochromatic obj. N. A. 1: 30, combined with compensating ocular 12; except *fig. 16*, which was drawn with compensating ocular 18.

FIGS. 49–65, *Empusa sciarae*. All ×1500, except fig. 64, which is ×2250.

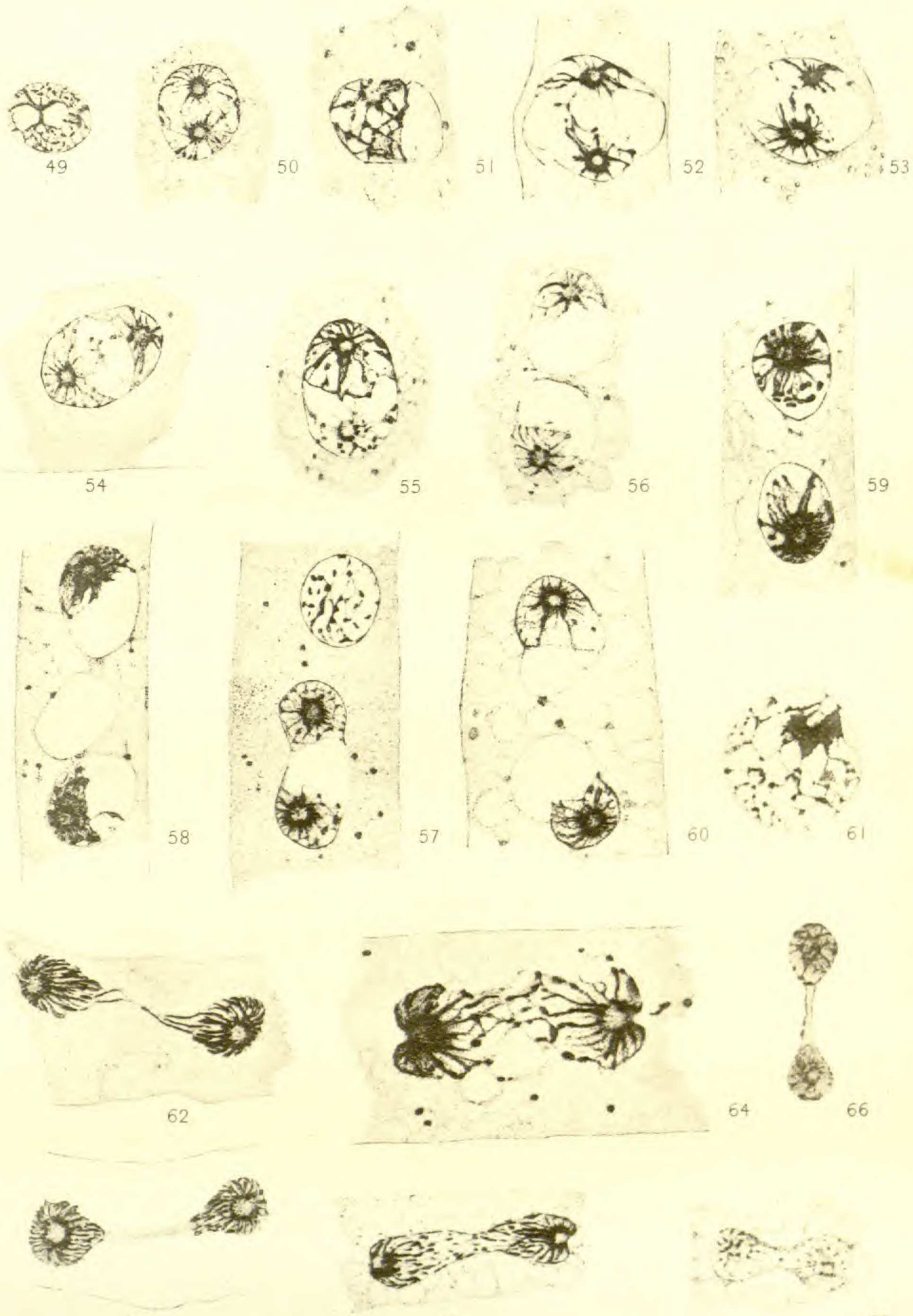
FIG. 49. Probably an early prophase of division.

FIG. 50. A considerably advanced stage of division.

FIG. 51. A poorly differentiated preparation in which the karyolymph has accumulated at one side of the dividing nucleus.

FIG. 52. A thin section of an anaphase.

FIG. 53. A similar preparation.



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